Improved Application of RNAModMapper – an RNA Modification Mapping Software Tool – for Analysis of Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) Data

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Supplemental Information

Supplemental Equations S1-S4

Supplemental Figures S1-S56

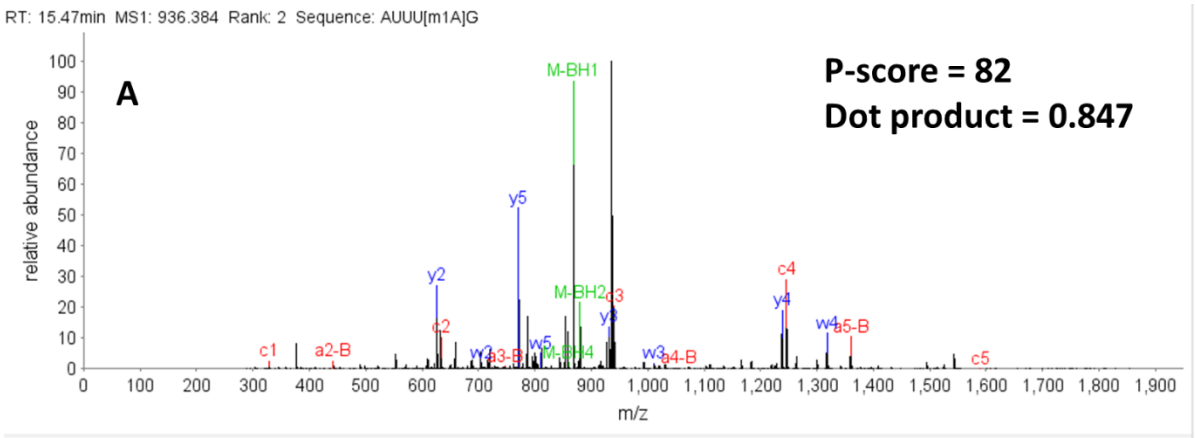
Equation 1: $$P(n, p, N) = \sum_{k=n}^{N} \binom{N}{k} p^k (1-p)^{N-k}$$

Equation 2: $$S(P) = \frac{100^{*}\log_{10}(P)}{N^{*}\log_{10}(p)}$$

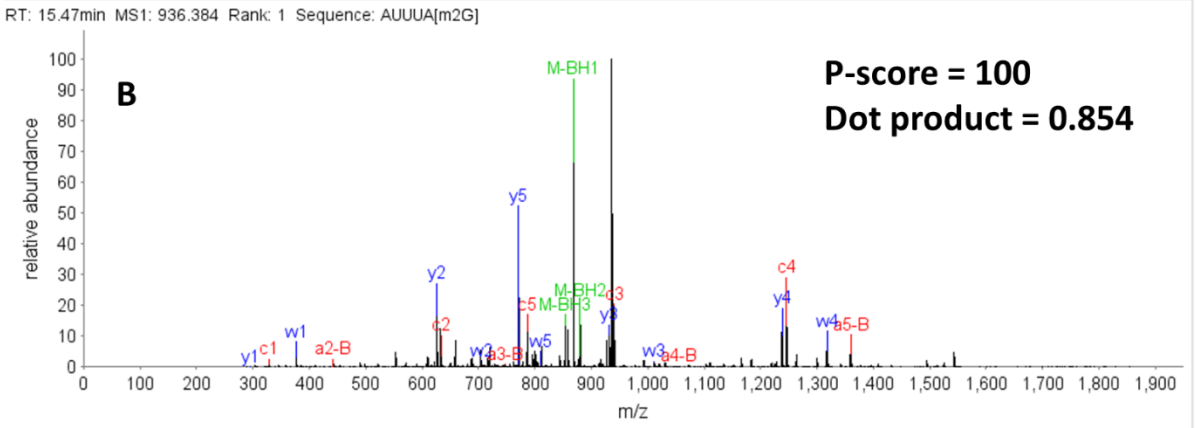
Equation 3: $$S(P_T) = [0.7^{*}S(P_{c/y})] + [0.3^{*}S(P_{a-B/w})]$$

Equation 4: $$\mathrm{DP} = \frac{\sum I_{\text{observed}} \times I_{\text{reconstructed}}}{\sqrt{\sum I^2_{\text{observed}} \times \sum I^2_{\text{reconstructed}}}}$$

Supplemental Figure S1. Sequence alignments obtained during fixed position mapping for ion-trap CID experiments.

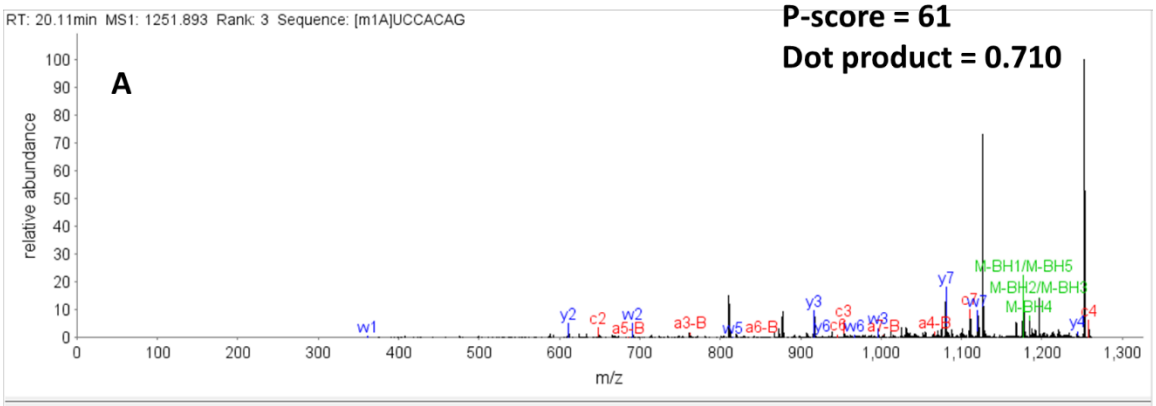


#	a-B	Error(Da)	c	Error(Da)	Oligo	w	Error(Da)	y	Error(Da)	#	#	Base loss	M-BH	Error(Da)	Neutra...	M-N	Error(Da)
5'					OH					5'	1	A	868.071	-0.541			
1			328.061	0.017	A					6	2	U	880.142	0.016			
2	442.088	0.012	634.05	-0.019	U	810.983	-0.109	771.041	-0.068	5	3	m1A	861.604	--			
3	747.187	-0.914	940.065	-0.029	U	1318.093	0.926	1238.127	0.926	4	4	G	860.193	-0.422			
4	1054.044	-0.082	1246.073	-0.046	U	1012.093	0.951	932.133	0.957	3							
5	1360.094	-0.057	1589.273	0.086	m1A	705.013	-0.104	625.153	0.002	2							
6					G	362.049	--	282.083	--	1							
3'					OH					3'							

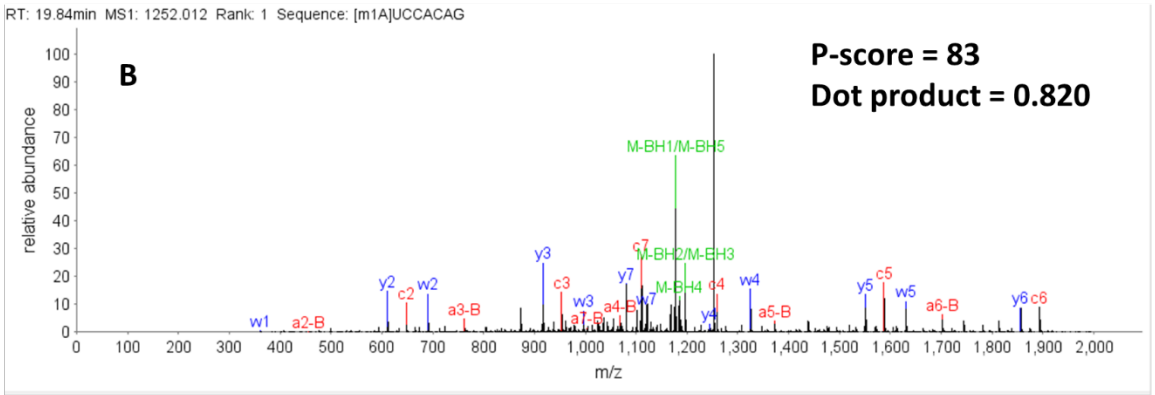


#	a-B	Error(Da)	c	Error(Da)	Oligo	w	Error(Da)	y	Error(Da)	#	#	Base loss	M-BH	Error(Da)	Neutra...	M-N	Error(Da)
5'					OH					5'	1	A	868.071	-0.541			
1			328.061	0.017	A					6	2	U	880.142	0.017			
2	442.088	0.012	634.05	-0.019	U	810.983	-0.109	771.041	-0.067	5	3	m2G	853.052	-0.554			
3	747.187	-0.914	940.065	-0.029	U	1318.093	0.927	1238.127	0.927	4							
4	1054.044	-0.082	1246.073	-0.046	U	1012.093	0.952	932.133	0.958	3							
5	1360.094	-0.057	787.09	0.008	A	705.013	-0.103	625.153	0.003	2							
6					m2G	376.043	-0.021	295.95	-0.148	1							
3'					OH					3'							

Supplemental Figure S2. Examples of (A) incorrectly and (B) correctly interpreted spectra for digestion product AUUUA[m^{2}G] acquired using ion-trap CID. Both interpretations were given using standard processing parameters.

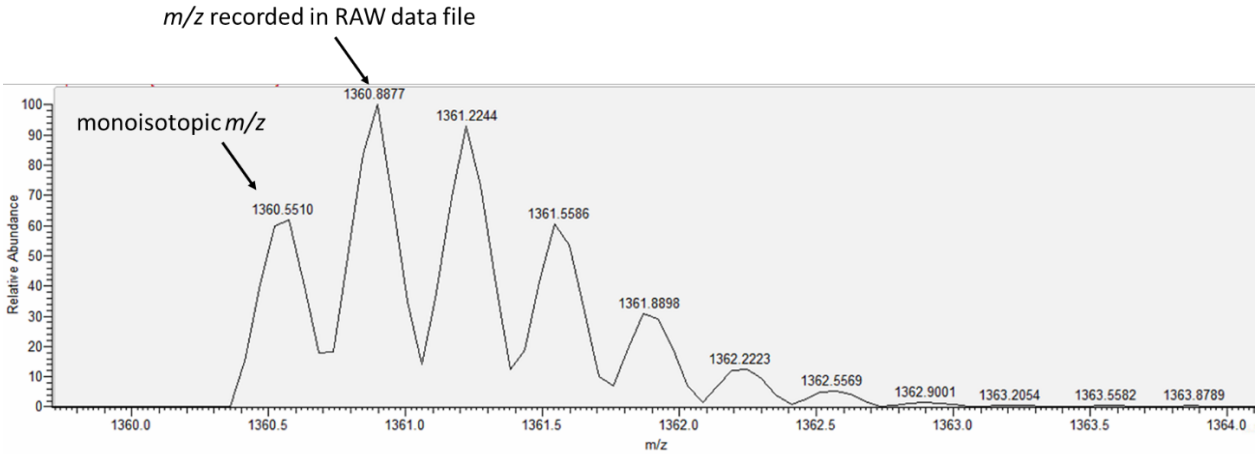


#	a-B	Error(Da)	c	Error(Da)	Oligo	w	Error(Da)	y	Error(Da)	#	#	Base loss	M-BH	Error(Da)	Neutral...	M-N	Error(Da)
5'					OH					5'	1	m1A	1177.025	-0.642			
1			342.06	--	m1A					8	2	U	1196.598	0.41			
2	456.092	--	647.981	-0.104	U	1119.914	-0.233	1080.807	0.643	7	3	C	1196.598	-0.082			
3	762.114	-0.003	953.11	-0.016	C	967.237	0.103	927.076	-0.075	6	4	A	1184.105	-0.57			
4	1067.028	-0.13	1258.069	-0.098	C	815.103	0.49	1550.269	--	5	5	G	1177.025	0.348			
5	685.104	-0.492	1587.219	--	A	1325.194	--	1245.166	-0.062	4							
6	851.1	0.978	945.931	0.305	C	996.042	-0.1	916.163	-0.013	3							
7	1002.6	-0.042	1110.098	-0.054	A	690.99	-0.111	611.033	-0.102	2							
8					G	362.012	-0.037	282.083	--	1							
3'					OH					3'							

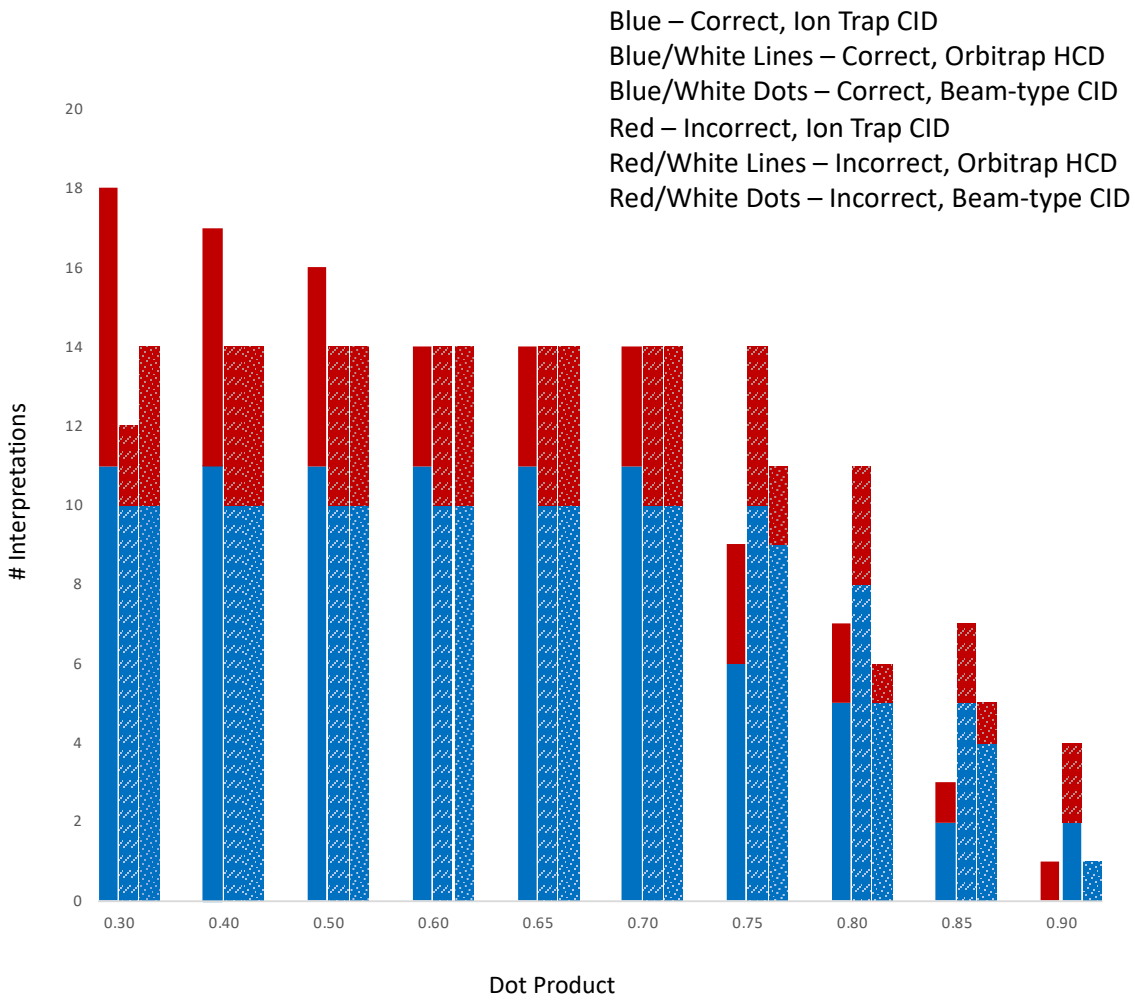


#	a-B	Error(Da)	c	Error(Da)	Oligo	w	Error(Da)	y	Error(Da)	#	#	Base loss	M-BH	Error(Da)	Neutral...	M-N	Error(Da)
5'					OH					5'	1	m1A	1177.334	-0.333			
1			342.06	--	m1A					8	2	U	1196.686	0.498			
2	456.321	0.229	648.128	0.043	U	1120.771	0.624	1080.674	0.51	7	3	C	1196.686	0.006			
3	762.108	-0.009	953.112	-0.014	C	1935.276	--	1855.264	-0.046	6	4	A	1184.615	-0.06			
4	1067.196	0.038	1258.072	-0.095	C	1630.129	-0.106	1550.177	-0.092	5	5	G	1177.334	0.657			
5	1372.094	-0.105	1587.109	-0.11	A	1325.121	-0.073	1245.174	-0.054	4							
6	1701.124	-0.127	1892.225	-0.035	C	996.191	0.049	916.197	0.021	3							
7	1002.661	0.019	1110.004	-0.148	A	691.051	-0.05	611.099	-0.036	2							
8					G	362.101	0.052	282.083	--	1							
3'					OH					3'							

Supplemental Figure S3. Examples of (A) incorrectly and (B) correctly interpreted spectra for digestion product [m^{1}A]UCCACAG acquired using ion-trap CID. Both interpretations were given using standard processing parameters.

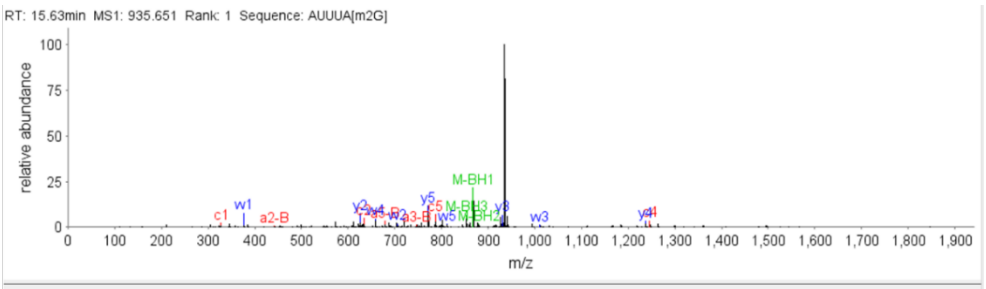


Supplemental Figure S4. Zoomed MS spectrum of precursor ion obtained digestion product A[Cm]U[Gm]AA[yW]AU[m^{5}C]UG, where ^{13}C isotope peak is more abundant than ^{12}C monoisotopic peak. The *m/z* value for the ^{13}C peak is stored in the RAW data file, requiring the precursor tolerance to be increased from 0.06 to ≥ 0.33.



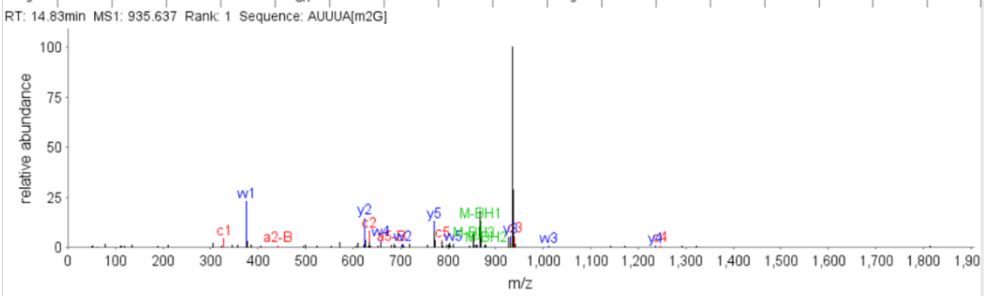
Supplemental Figure S5. Number of correct and incorrect interpretations using variable position sequence mapping for high resolution CID, HCD, and beam-type CID are different dot product thresholds.

A



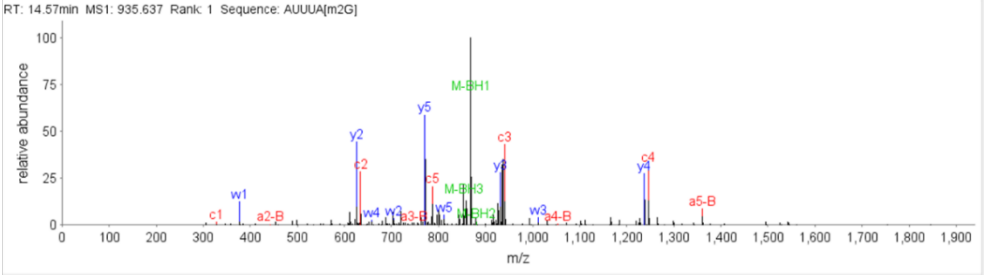
#	a-B	Error(Da)	c	Error(Da)	Oligo	w	Error(Da)	y	Error(Da)	#	#	Base loss	M-BH	Error(Da)	Neutra...	M-N	Error(Da)
5'					OH					5'	1	A	868.126	-0.486			
1			328.028	-0.016	A					6	2	U	879.633	-0.492			
2	442.036	-0.04	634.068	-0.001	U	811.106	0.014	771.123	0.015	5	3	m2G	853.117	-0.489			
3	747.106	-0.995	940.094	--	U	658.089	0.01	1237.246	0.046	4							
4	1054.126	--	1246.152	0.033	U	1011.171	0.03	931.198	0.023	3							
5	679.572	0.0	787.094	0.012	A	705.125	0.009	625.153	0.003	2							
6					m2G	376.052	-0.012	296.098	--	1							
3'					OH					3'							

B



#	a-B	Error(Da)	c	Error(Da)	Oligo	w	Error(Da)	y	Error(Da)	#	#	Base loss	M-BH	Error(Da)	Neutral...	M-N	Error(Da)
5'					OH					5'	1	A	868.612	0.0			
1			328.045	0.001	A					6	2	U	880.133	0.008			
2	442.079	0.003	634.072	0.003	U	811.101	0.009	771.111	0.003	5	3	m2G	853.604	-0.002			
3	748.101	--	940.095	0.001	U	658.08	0.001	1237.194	-0.006	4							
4	1054.126	--	1246.117	-0.002	U	1011.157	0.016	931.176	0.001	3							
5	679.572	0.0	787.08	-0.002	A	705.121	0.005	625.153	0.003	2							
6					m2G	376.067	0.003	296.098	--	1							
3'					OH					3'							

C



#	a-B	Error(Da)	c	Error(Da)	Oligo	w	Error(Da)	y	Error(Da)	#	#	Base loss	M-BH	Error(Da)	Neutral ...	M-N	Error(Da)
5'					OH					5'	1	A	868.61	-0.002			
1			328.045	0.001	A					6	2	U	880.129	0.004			
2	442.074	-0.002	634.07	0.001	U	811.095	0.003	771.111	0.003	5	3	m2G	853.606	0.0			
3	748.102	0.001	940.095	0.001	U	658.081	0.002	1237.202	0.002	4							
4	1054.124	-0.002	1246.12	0.001	U	1011.145	0.004	931.177	0.002	3							
5	1360.152	0.001	787.083	0.001	A	705.119	0.003	625.152	0.002	2							
6					m2G	376.066	0.002	296.098	--	1							
3'					OH					3'							

Supplemental Figure S6. Tabular output, including calculated mass errors, for interpreted MS/MS spectra provided in Figure 5. (A) beam-type CID (B) HCD and (C) ion-trap CID for digestion product AUUUA[m^{2}G].